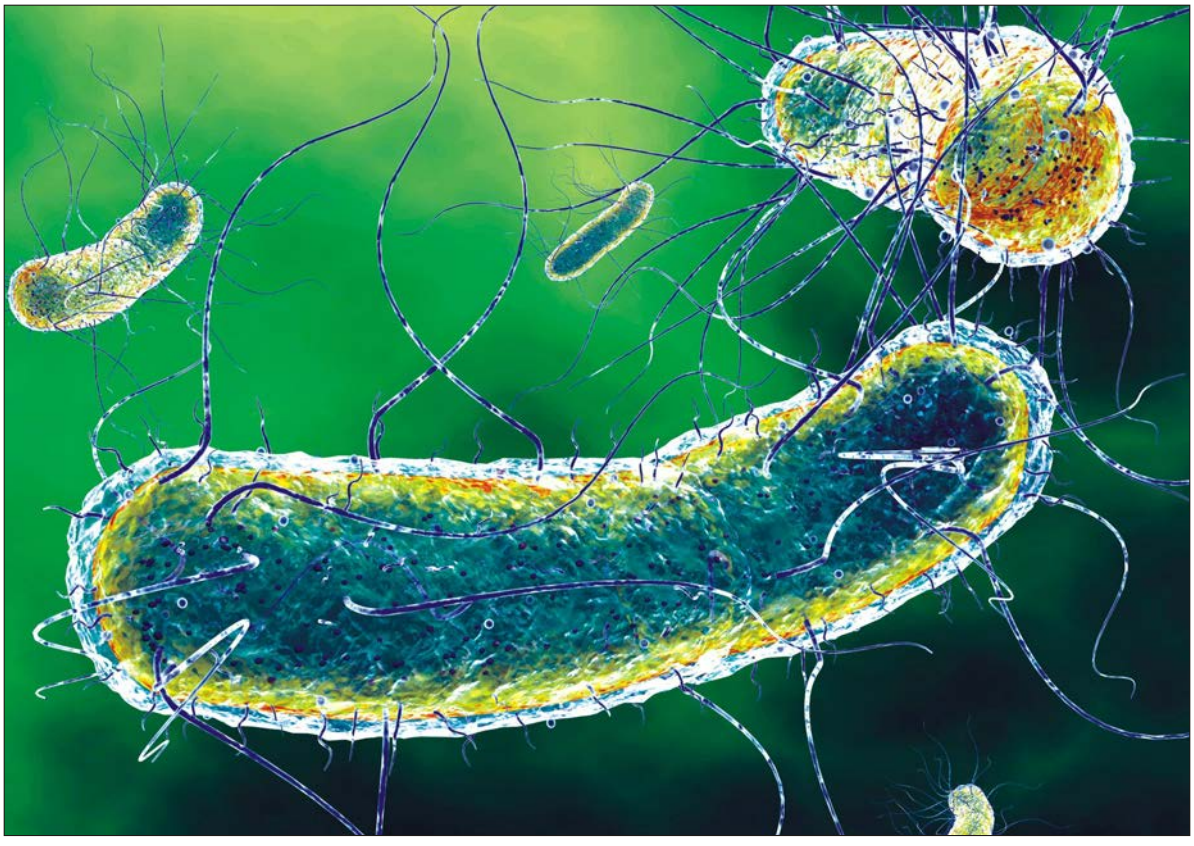

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Antibiotics Resistance and Plasmid Profile of *Staphylococcus aureus* from Wound Swabs in Port Harcourt Nigeria

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ABSTRACT

Staphylococcus aureus are organisms that have been detected as the major pathogens in hospital and community settings that are responsible for infections and their resistance to commonly used antibiotics has become worldwide concern. The indiscriminate use of antibiotics has led to the presence of drug resistant organisms. A total of 100 wound swabs were obtained from two tertiary hospitals in Port Harcourt, Nigeria and screened for *S. aureus*. This was done by streaking the swab sticks on sterile mannitol salt agar plates, positive growths were identified using conventional methods based on morphological and biochemical characteristics. Identified *S. aureus* was subjected to antibiotics susceptibility test using Kirby Bauer disc method. Plasmid profiling was also carried out on 30 randomly selected *S. aureus* using gel electrophoresis. Out of the 100 wound swabs examined, ninety-two were positive for *S. aureus*. Antibiotic susceptibility testing showed that *S. aureus* was 100% resistant to caftaxidim, ceftriazone, ofloxacin and ceptazdime. *Staphylococcus aureus* was sensitive to ofloxacin and gentamicin (88%), erythromycin (84%) and augumentin (82%). Plasmid profile result for *S. aureus* revealed single plasmid sizes of 2027, 2322 4361 and 23130 bp, respectively while double plasmid size was observed in one isolate. The determination of antibiotic sensitivity pattern and plasmid profiling of *S. aureus* isolated from wound will assist in the preliminary treatment of wound infections.

Key words: Antibiotics, plasmid profile, resistance, *Staphylococcus aureus,* wound

INTRODUCTION

*Staphylococcus aureu*s is a facultative anaerobe, non-motile gram positive bacteria that occur singly in pairs (Akpaka *et al*., 2006). *Staphylococcus* species are known to be responsible for a wide number of diseases both in humans and animals. *Staphylococcus aureus* frequently colonizes the human skin, skin glands and mucous membrane of warm blooded animals. *Staphylococcus aureus* is present in about 25-30% of humans especially adults (Lowy, 1998; Akpaka *et al*., 2006; Haque *et al*., 2011; Den Heijer *et al.,* 2013). Most times they can exist as normal flora of the skin, however if there is a break in the skin from a wound or surgery, or if there is a suppression of a person's immune system, then the presence of *S. aureus* in the skin can cause an infection (Koneman *et al*., 1997). *Staphylococcus aureus* lives mostly on mucosal surfaces (Chiquet *et al*., 2007) and it is frequently involved in numerous hospital acquired infections (Chiquet *et al*., 2007). The mucoid strain observed in *S. aureus* is due to the capsule which enhances virulence (Alberich and Herbarth, 2001; Chiquet *et al*., 2007; Jensen and Lyon, 2009). *Staphylococcus aureus*

is responsible for a broad spectrum of human and animal diseases, not just skin infections but severe diseases in humans and animals such as pneumonia and endocarditis among others (Dar *et al*., 2006). The genes that are responsible for antibiotic resistance in *S. aureus* are usually found on the plasmids or some other structures similar to plasmids (Sambrook *et al*., 1989). There has been an increase on drug resistance of *S. aureus* to most antibiotics in recent times, only few antibiotics are available for most of the staphylococci which are glycopeptides antibiotic such as vancomycin and teicoplanim (Chiquet *et al*., 2007; Dar *et al*., 2006). Infections by some species of staphylococci are difficult to treat because of frequency of multiple antibiotic resistant strains.

The genes for antibiotic resistance in *S. aureus* are located on the plasmid, these plasmids contain resistant genes against a number of antimicrobial agents (Simpson *et al*., 2007). Studies carried out to determine the role of plasmids on antibiotic resistance has been useful in determining the characteristics of plasmids in bacteria. Plasmids can be transferred from one close bacterium to another horizontally, while for bacterium that is distant from one another plasmids can be transferred phylogenetic (Dale and Park, 2004). These two modes of transfer of plasmids might be responsible for the spread of antibiotic resistance genes in the environment (Dale and Park, 2004). This gives rise to the suspicion that there is a possibility that these resistant genes can be transferred from one bacterium to another (Imade *et al*., 2010). Plasmid profiling and restriction endonuclease analysis of plasmid DNA are among the molecular techniques that are currently being used in differentiating strains of bacteria isolates. Plasmid profiles have been useful in the epidemiological surveillance of disease outbreaks and in tracing antibiotic resistance (Liu *et al*., 1995; Dutta *et al*., 2002; Tayfour *et al.,* 2005).

The present study was carried out to determine the antibiotic resistant pattern and plasmid profile of *S. aureus* obtained from wound swabs.

MATERIALS AND METHODS

Source of specimen: A total of 100 wound swabs were obtained from patients attending two hospitals within Port Harcourtfrom from October 2013 to February 2014. Wound swabs were immediately transported to the University of Port Harcourt medical laboratory for isolation and characterization. After isolation and identification the isolates were taken to Nigerian Institute for Medical Research for plasmid profiling.

Isolation and identification of *S. aureus*: The swabs were aseptically cultured on mannitol salt (Oxoid) agar plates and incubated at 37°C for 24-48 h and examined for pure distinct colonies. The identification of the *Staphylococcus* isolates was done following standard microbiological technique which involves morphological, cultural characteristic, biochemical characteristics and coagulase test. The isolates were sub-cultured into nutrient agar (Oxoid) slant and incubated at 37°C for 24-48 h and later stored in the refrigerator for further use (Cowan *et al*., 2003; Cheesbrough, 2006; CLSI., 2011).

Antimicrobial susceptibility pattern of *Staphylococcus* isolates: Antibiotic susceptibility pattern was carried out on 92 isolates using Kirby Bauer disc diffusion method. From each slant culture of isolates, five representative colonies were touched with a sterile wire loop and suspended in sterile nutrient broth (Oxoid). Each isolate suspension was adjusted to 1×10^8 densities equal to the 0.5 McFarland standards before inoculation. From the nutrient broth 0.1 mL of the isolate was

surface spread evenly on Muller-Hinton Agar (MHA, Oxoid)) plates. Sensitivity discs were picked with a pair of sterile forceps and placed on the plate which was incubated aerobically at 37°C for 24 h (CLSI., 2011). The antibiotics used were as follows: ceftazidime (CAZ) 30 µg, gentamicin (GEN)10 µg, cefuroxime (CRX) 30 µg, ceftriaxone (CTR) 30 µg, cloxacillin (CXC) 5 µg, ofloxacin (OFL) 5 µg, erythromycin (ERY) 30 µg, amoxicillin/clavulanate (AUG) 30 µg.

Plasmid profile of *S. aureus* isolates: Out of the 92 identified *Staphylococcus* isolates obtained, plasmid profiling was carried out on 30 randomly selected *S. aureus* isolates. Plasmid extraction was carried out based on the methods of Molina-Aja *et al*. (2002) with slight modifications. In brief, a single bacterial colony was picked up and grown in 5.0 mL of Muller Hilton broth in an eppendorf tube and centrifuged at 10,000 rpm for 2 min. The cell pellets obtained were re-suspended in 150 µL EDTA-Tris buffer and vortexes to mix. This was followed by the addition of 175 µL of 2% SDS and 175 µL of 0.4N NaOH. The tube was mixed vigorously, 250 µL of cold 5 M potassium acetate was added vigorously, the tube was centrifuged at 12,000 rpm for 5 min and the supernatant was transferred to a sterile 1.5 mL eppendorf tube and equal volume of cold isopropanol was added. After inverting gently, the mixture was immediately centrifuged at 12,000 rpm for 10 min and the DNA pellet was washed with 650 µL of cold (4°C) 70% ethanol by centrifuging at 12,000 rpm for 15 min. The supernatant was discarded and the pellet was dried for 30 min and re-suspended in 40 µL of sterile deionized water. Agarose gel was prepared and poured into electrophoresis tank and allowed to solidify. The sample 15 µL was mixed with 2 µL of the loading dye was loaded into wells. Gels were visualized and photographed using digital photo documentation system (Clinix, Japan) (Sambrook *et al*., 1989; Molina-Aja *et al*., 2002).

Calculation of molecular weight fragments: The fragment bands observed were directly compared with the molecular weight marker bands Hind III digest of Lambda phage from Roche Diagnostics Germany) which has eight distinct visually observed bands with molecular weight 1820, 21140, 23130, 9416, 6557, 4361, 2322 and 2027 bp.

RESULTS

Out of the 100 wound swabs collected, 92 of them were positive for *S. aureus*. Identified *S. aureus* were subjected to antibiotic sensitivity test using the disc diffusion method. Percentage of resistance and sensitivity of the isolates to antibiotics was shown in Table 1. *Staphylococcus aureus* was 100% resistant to ceftazidime followed by ceftriaxone (88.04%). Most of the isolates were highly susceptible to ofloxacin (95.65%) followed by gentamicin (88%), erythromycin (84%) and augumentin (82%) as shown in Fig. 1.

Table 1: Percentage of resistance and sensitive *Staphylococcus aureus* to antibiotics

Antibiotics	Isolates	Resistant No.	Intermediate No.	Susceptible No.	Resistant(%)	Intermediate (%)	Susceptible (%)
CAZ	92	92	-	-	100.00	0	0
CRX	92	19	-	73	20.57	-	79.34
GEN	92	4	-	88	4.30	-	95.65
CTR	92	81	-	11	88.04	-	11.95
ERY	92	8	-	84	8.69	-	91.30
CXC	92	55	17	20	59.78	18.47	21.50
OFL	92	-	4	88	-	4.35	95.65
AUG	92	8	2	82	8.69	2.17	89.13

CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxacillin, OFL: Ofloxacin, AUG: Amoxicillin/Clavulinate

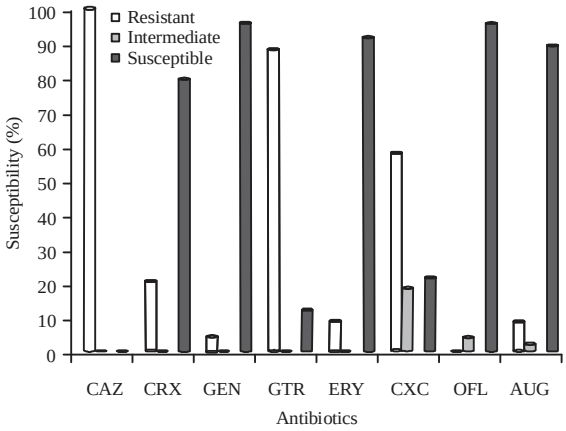

Fig. 1: Percentage susceptibility pattern of *Staphylococcus aureus* to antibiotics

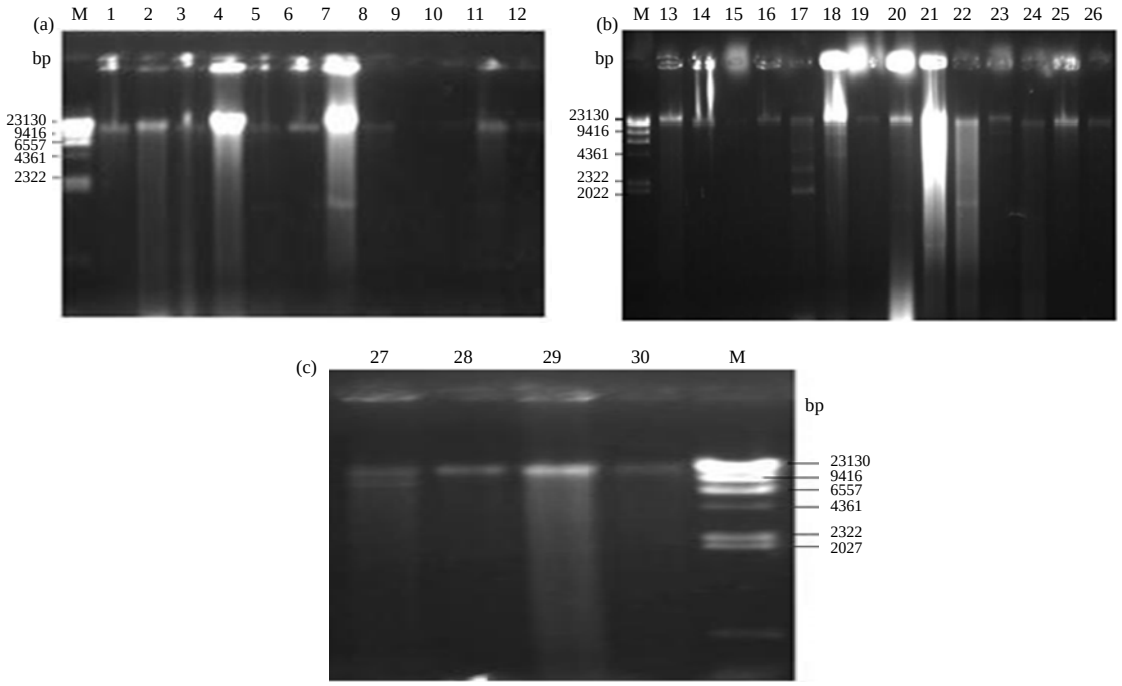

Fig. 2(a-c): Plasmid profile of isolate in (a) Lane 1-12, (b) Lane 13-26 and (c) 27-30

Thirty isolates were randomly selected from the 92 *S. aureus* isolates for plasmid profiling. Results of plasmids are showed in Fig. 2a-c. The plasmid profile result for *S. aureus* revealed that *S. aureus* possessed mostly single plasmid, just a few had double plasmid size. In Fig. 2 isolates in lane 9 and 10 does not possess plasmid. The plasmid size for all the isolates in Fig. 2 is 23130 bp.

Figure 2b shows the plasmid profile of *Staphylococcus aureus* isolates in lane13-26. The isolate in lane15 does not possess plasmid while isolate 17 had four plasmid sizes of 2027, 2322, 4361and 23130 bp.

Figure 2c shows the plasmid profile of isolates in lane 27-30. Isolate 27 had double plasmid of 23130 and 9416 bp while isolate 28, 29 and 30 had plasmid size of 23130 bp.

DISCUSSION

The study was carried out to determine the antibiotic resistance and plasmid profile of *S. aureus* from wound swab. The antimicrobial susceptibility pattern was determined for all isolates. In this study, the frequency of *S. aureus* isolates was 92%. The frequency is higher than reports from previous studies from various clinical swabs (Akpaka *et al*., 2006; Rajaduraipandi *et al*., 2006; Al-Hamdani and Hamad, 2012). Their studies reported a frequency of *S. aureus* of 47.3% from various clinical swabs. All *S. aureus* isolates tested in this study were completely resistant to ceftazidime (100%) and ceftriaxone (88.04%) antibiotics. A similar result was also reported in a study where complete resistance of *S. aureus* to ceftazidime was observed while the isolates in our study were susceptible to erythromycin and gentamicin (Akpaka *et al*., 2006). Our study is also in agreement with previous study where result showed gentamicin and erythromycin was more effective in the management of *S. aureus* from surgical wound in that locality (Idighr *et al*., 2012). In the present study, *S. aureus* susceptibility to ofloxacin and gentamicin was 95.65%. This result is in agreement with reports from Nwankwo and Nasiru (2011) who reported 76.6% susceptibility to ofloxacin and 73.4% for gentamicin. This also compares favorably with results from other researchers. Previous studies have shown 100% resistance of *S. aureus* to AUG which is in contrast to the finding of this work. Similar work done carried out showed 6% resistance to gentamicin and resistance rate of 10.4% to ofloxacin (Rajaduraipandi *et al*., 2006). A study carried out by Stanley *et al.* (2013) on the antimicrobial susceptibility of *S. aureus* from isolated pregnant women showed that many were only moderately susceptible to tested antibiotics. In their study levofloxacin, ceftriaxone, clindamycin and erythromycin all appeared in decreasing order (Stanley *et al.,* 2013). Their work was in agreement with the study carried out by Imade *et al*. (2010), where results obtained suggested ceftriaxone, ciprofloxacin, augumentin, ofloxacin and gentamicin as drugs of choice for *S. aureus*.

In most developing countries antimicrobial resistance is an aspect of great concern thus the need for further research. Plasmid profile studies are widely used for epidemiological studies and the role of plasmids in drug resistance. The wide spread of antibiotics resistance observed in isolates that possessed plasmids in the present study can be associated to the increased and abusive use of antibiotics (Liu *et al*., 1995; Ombui *et al*., 2000; Davis and Amabile-Cuevas, 2003). Most of the *S. aureus* in the present study showed similar antibiotic resistance pattern, while different plasmid sizes was observed in most of the isolates. This disparity can be due to R- plasmids of different sizes which are also responsible for the presence of multiple resistances. *Staphylococcus aureus* can acquire genetic elements from other bacteria. Large mobile genetic elements appear to encode both antibiotic resistant factors and proteins that are responsible for increase in virulence thus giving the organism the ability to adapt to the selective pressure of antibiotics (Alberich and Herbarth, 2001; Ojo *et al*., 2014). Plasmid profile analysis of isolates makes it possible to evaluate the dependence between antibiotic susceptibility and the presence of plasmids with respect to a particular isolate (Ojo *et al*., 2014). It can be said that resistance mediated by plasmid which has been observed in the various studies may be responsible for the differences observed in treatment by clinicians (Alberich and Herbarth, 2001; Ojo *et al*., 2014).

CONCLUSION

In this study, it was observed that *S. aureus* was a major pathogenic agent prevalent in wound. The high level of resistance could be associated with exposure of these drugs to isolates which may have enhanced development of resistance. There is a high level of antibiotic abuse in developing

countries such as Nigeria arising from self-medication associated with inadequate dosage and failure to comply with treatment and availability of antibiotics to consumers across the counters with or without prescription.

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